that epigenetic states and thereby transcriptomes are altered in a directed and heritable way. The work presents an interesting example of the Lamarckian paradigm (Martienssen, 2008; Koonin and Wolf, 2009), in which the environment directs evolution, and induced changes in the epigenetic state may in turn create variations in gene expression to be explored, selected for, and coopted through the generations.

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Stuck in the Middle: Drugging the Ubiquitin System at the E2 Step

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DOI 10.1016/j.cell.2011.06.002

The discovery of a small-molecule allosteric inhibitor of the CDC34 ubiquitin-conjugating enzyme (E2) by Ceccarelli et al. raises the possibility that it will be generally feasible to selectively inhibit ubiquitin transfer at this central step in the ubiquitin pathway.

There is significant interest in targeting the ubiquitin proteasome system (UPS) with small molecules for treatment of diseases such as cancer and neurodegeneration (Bedford et al., 2011). In this pathway, ubiquitin is transferred to substrates through cycles of an E1-activating enzyme-E2-conjugating enzyme-E3 ubiquitin ligase cascade, leading to production of a polyubiquitin chain covalently attached to the substrate that targets it for rapid degradation by the proteasome (Figure 1A). Though molecules targeting the E1 class of enzymes as well as the proteasome are already in the clinic or in trials, the development of inhibitors of components in the middle of the pathway have lagged behind. This is despite the fact that E2s and E3s control the substrate specificity and the type of ubiquitin linkage formed and therefore potentially represent more specific targets for small-molecule-based therapy. In this issue of *Cell*, Ceccarelli et al. (2011) report the identification of the first inhibitor of an E2 enzyme, in this case the human E2 CDC34 (also called UBE2R1), which plays a critical role in the elimination of cell-cycle regulatory proteins by the proteasome (Skaar and Pagano, 2009). Unexpectedly, the inhibitor acts allosterically by binding to a pocket distant from the active site. This study indicates that it is possible to develop a highly selective inhibitor of an E2 enzyme and raises the possibility of targeting other E2s in a similar manner.

The success of proteasome inhibitors clinically has fueled the interest in developing inhibitors that block specific E1-E2-E3 pathways (Bedford et al., 2011). By targeting one or a small number of E3-dependent processes, the effects on overall protein homeostasis may be restricted to the precise pathway intended. CDC34 is one of approximately two dozen ubiquitin E2s in mammals but is unique in that it functions solely with one specific class of E3s: the cullin-RING ligase (CRL). Cullins employ a RING finger protein (RBX1 or RBX2), together with a conserved pocket in cullins themselves, to recruit ubiquitin-charged CDC34 (Deshaies and Joazeiro, 2009; Kleiger et al., 2009). CRL activity also requires modification of cullin by the ubiquitin-like NEDD8 protein, a process that is inhibited by a small-molecule inhibitor of the neddylation E1 enzyme (Figure 1B) (Bedford et al., 2011). As CRLs are responsible for controlling the turnover of cancer-related genes, including the cell-cycle inhibitor p27 (Skaar and Pagano, 2009), this class of E3s has received particular attention in cancer drug discovery.

To identify small-molecule inhibitors of p27 ubiquitination, Ceccarelli et al. employed a fully reconstituted p27 ubiquitination reaction involving E1, CDC34, the E3 $CRL1^{SKP2\text{-}CKS1}$, and p27 in a high-throughput screen, identifying CC0651 as a prime candidate for an inhibitor of this pathway. Given the number of components and the complexity of the process being examined in this assay, CC0651 could block one of numerous steps. Using a variety of related E3 CRL complexes and

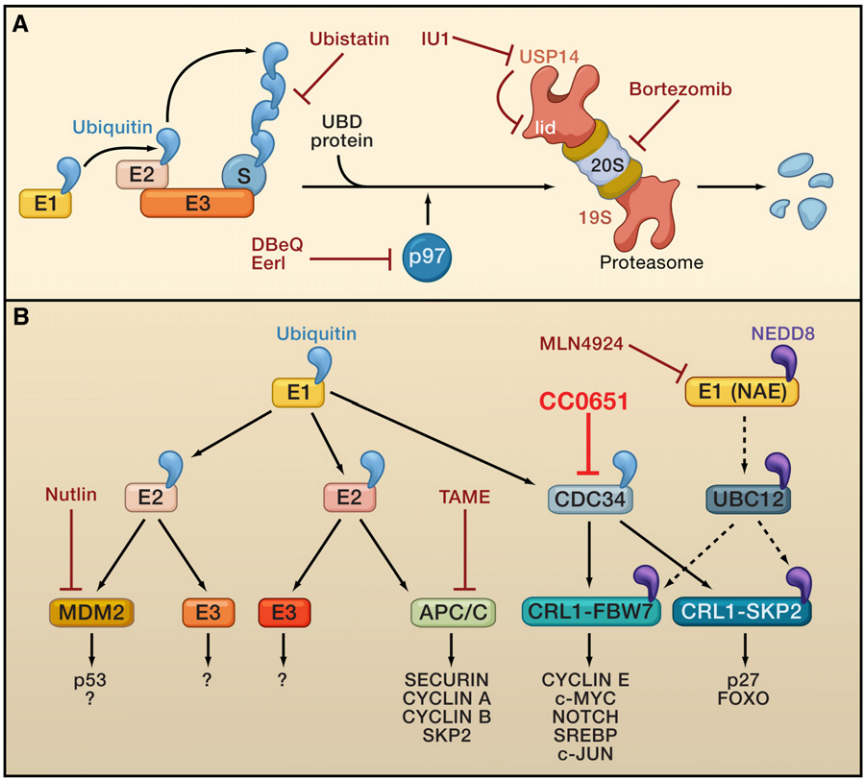

Figure 1. Hierarchical Nature of the Ubiquitin-Proteasome System and Sites of Intervention by Small Molecules

(A) Overview of the ubiquitin conjugation cascade showing the points at which Ubistatin, DBeQ, Eerl, the USP14 inhibitor IU1, and Bortezomib block the pathway.

(B) Scheme depicting the hierarchy of the ubiquitin cascade. E1 enzymes act at the top of the cascade to promote charging of multiple E2 enzymes with ubiquitin, which then work with various E3s to promote substrate ubiquitination, some of which are shown. The NEDD8 E1 (NEDD8-activating enzyme [NAE]), which is inhibited by MLN4924, works in the same way to activate UBC12-related E2s, which then transfer the ubiquitin-like protein NEDD8 to the cullin subunit of CRLs, resulting in their activation. The MDM2 E3 is inhibited by Nutlin, and the APC/C E3 is inhibited by TAME. CC0651 is a specific inhibitor of CDC34. Two specific CRL complexes with differing substrate specificity are shown. UBD, ubiquitin-binding domain.

their substrates, all of which employ CDC34, as well as non-CRL-based E3s that employ distinct E2s, Ceccarelli et al. pinpointed the likely target of CC0651 as CDC34 itself. As expected for an inhibitor of CDC34, CC0651 and related derivatives caused accumulation of p27 and cyclin E in cells and inhibited proliferation in a CDC34-dependent manner.

The specificity of CC0651 for human CDC34 is remarkable. CC0651 inhibited ubiquitination of a yeast CRL-based E3 in the context of human CDC34, but not the same E3 in conjunction with yeast Cdc34p. CC0651 had no ability to inhibit any other E2/E3 pairs tested and was even inactive in assays using CDC34B (UBE2R2), the closest relative of CDC34. To understand the basis of this selectivity, Ceccarelli crystallized CDC34 with CC0651 and found that it binds a pocket on the opposite side of a central helix that buttresses the active site. Interestingly, this pocket is not fully formed in the apoCDC34 structure but becomes apparent only upon small-molecule binding, indicative of an induced fit mechanism. CC0651 interacts with its binding site largely through hydrophobic interactions plus a single hydrogen bond. Together, these interactions help CC0651 inhibit hCDC34 with a potency of 1.9 μM. Whether this binding pocket affords opportunities for additional interactions to drive potency into the nanomolar range is an important question that will impact the success of CC0651 as a therapeutic developmental candidate.

Given that CC0651 does not directly contact the active site of the enzyme, how does the inhibitor perturb ubiquitin transfer to substrates? Structural analysis suggested several possibilities. CC0651 binding to CDC34 caused the helix bearing the catalytic cysteine to be displaced by 2.0 Å. In addition, the interaction surfaces with the donor ubiquitin (to be discharged from the enzyme) and with a surface that interacts with E1 and E3 were perturbed (Ceccarelli et al., 2011). However, biochemical assays revealed that E1 and E3 interactions remained intact in the presence of the inhibitor. Instead, CC0651 specifically perturbed the ability of CDC34 to promote ubiquitin transfer and chain formation on substrates, turning its conjugating activity on itself and free ubiquitin. Though CC0651 is clearly not acting as an active site-directed inhibitor, a complete understanding of its mechanism will require further enzymology as well as structural characterization in combination with the ligase and its substrates.

The last few years have seen significant advances in the identification of small molecules that block various steps in the UPS, in addition to E1s and the proteasome. These include inhibitors targeting distinct E3s [$CRL1^{Cdc4p}$ and the anaphase-promoting complex/cyclosome (APC/c)], small molecules that block ubiquitin recognition by binding to ubiquitin chains, inhibitors of USP14 (a negative regulator of the proteasome), and inhibitors of the p97 ATPase that is involved in remodeling ubiquitinated proteins to enable delivery to the proteasome (Fiebiger et al., 2004; Orlicky et al., 2010; Zeng et al., 2010; Verma et al., 2004; Chou et al., 2011; Bedford et al., 2011) (Figures 1A and 1B). An exciting implication of the new work is that it might be possible to target other E2s selectively in the future. Ceccarelli et al. suggest that other E2s also have partially preformed pockets in the same position as found in CDC34. However, whether these pockets can breathe in the same way as observed for CDC34 remains an open question. Furthermore, if such pockets do indeed breathe, it remains unclear whether they differ substantially enough from CDC34 or contain enough structural complexity to afford development of highly selective and potent inhibitors. In order to find

them, however, screening of completely reconstituted ubiquitylation cascades may be necessary, as simple E1-E2 thioester assays would not have identified the CDC34 inhibitor found here. The identification of CC0651 is an exciting finding that sets the stage for the discovery of new E2 inhibitors, but only further work will reveal whether blocking ubiquitylation in the middle of the pathway will be better than blocking it at either end.

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The Pessimist's and Optimist's Views of Adult Neurogenesis

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DOI 10.1016/j.cell.2011.06.011

The reports by Bonaguidi et al. (in this issue of *Cell*) and Encinas et al. (in *Cell Stem Cell*) come to differing conclusions about whether and how the proliferation of radial glia-like stem cells of the adult hippocampus impacts their long-term potential for neurogenesis.

Adult neurogenesis had remained a footnote in neurobiology until the discovery of neural stem cells in the 1990s, which offered an explanation of where new neurons of the adult hippocampus and olfactory bulb might originate from. It was later discovered that the stem cells of the adult neurogenic regions have astrocytic properties and a morphology like radial glia. In the dentate gyrus of the hippocampus, these cells have a prominent process that branches out into the molecular layer. The question then arose of whether and how cells with such elaborate radial morphology would be capable of self-renewal—not only by asymmetric division (in which one morphologically distinct daughter cell would be generated), but also by symmetric division (which would produce not one but two new radial cells). Linked to this question is the important problem of how the type and rate of self-renewal would affect the population of stem cells over time. Now, two reports (in *Cell* [Bonaguidi et al., 2011] and *Cell Stem Cell* [Encinas et al., 2011]) come to substantially differing conclusions about the ability of radial glia-like stem cells in the hippocampus to self-renew and thus their capacity for maintaining neurogenic potential throughout life (Figure 1).

In a meticulous study based on various transgenic reporter models in mice, Encinas and colleagues show that the radial-glia like type-1 cells (quiescent neural progenitors [QNP] in their nomenclature; Mignone et al., 2004) divide asymmetrically to give rise to intermediate progenitor cells (amplifying neural progenitors [ANP], or type 2 in our nomenclature; Kempermann et al., 2004). The authors never observed symmetric division, and over time, the QNP cells disappear from the subgranular zone of the dentate gyrus by differentiating into astrocytes, thereby drying out the source for more new neurons (Encinas et al., 2011). In the study by Bonaguidi and colleagues, published in this issue, the authors use transgenic mice to induce sparse labeling of precursor cells (including the amazingly sophisticated two-color MADM reporter; Zong et al., 2005) to address a similar question